CLAIMS

1. An α-glucan phosphorylase having improved thermostability, which is obtained by modifying a natural α-glucan phosphorylase,

wherein the natural α-glucan phosphorylase is derived from a plant, and

the α-glucan phosphorylase having improved thermostability has an amino acid residue which is different from that of the natural α-glucan phosphorylase in at least one position selected from the group consisting of:

a position corresponding to position 4 in a motif sequence 1L: H-A-E-F-T-P-V-F-S or a position corresponding to position 4 in a motif sequence 1H: H-A-Q-Y-S-P-H-F-S;

a position corresponding to position 4 in a motif sequence 2: A-L-G-N-G-G-L-G; and

a position corresponding to position 7 in a motif sequence 3L: R-I-V-K-F-I-T-D-V or a position corresponding to position 7 in a motif sequence 3H: R-I-V-K-L-V-N-D-V; and wherein

enzyme activity of the α-glucan phosphorylase having improved thermostability at 37°C, after heating in a 20 mM citrate buffer (pH 6.7) at 60°C for 10 minutes, is 20% or more of enzyme activity of the α-glucan phosphorylase having improved thermostability at 37°C, before heating.

2. The α-glucan phosphorylase having improved thermostability according to claim 1, wherein the natural α-glucan phosphorylase has an amino acid residue which is different from an amino acid residue of the natural α-glucan phosphorylase at a position corresponding to position 4 in the motif sequence 1L, or a position corresponding to position 4 in the motif sequence 1H, a position corresponding to

position 7 in the motif sequence 3L, or a position corresponding to position 7 in the motif sequence 3H.

3. The α-glucan phosphorylase having improved thermostability according to claim 1, wherein the amino acid sequence of the natural α-glucan phosphorylase has at least 50% identity with an amino acid sequence selected from the group consisting of: a position 1 to position 916 of SEQ ID NO: 2; position 1 to position 912 of SEQ ID NO: 4; position 1 to position 893 of SEQ ID NO: 6; position 1 to position 939 of SEQ ID NO: 8; position 1 to position 962 of SEQ ID NO: 10; position 1 to position 971 of SEQ ID NO: 12; position 1 to position 983 of SEQ ID NO: 14; position 1 to position 928 of SEQ ID NO: 16; position 1 to position 951 of SEQ ID NO: 18; position 1 to position 832 of SEQ ID NO: 20; position 1 to position 840 of SEQ ID NO: 22; position 1 to position 841 of SEQ ID NO: 24; position 1 to position 842 of SEQ ID NO: 26; position 1 to position 841 of SEQ ID NO: 28; and a position 1 to position 838 of SEQ ID NO: 30.

4. The α-glucan phosphorylase having improved thermostability according to claim 1, wherein the amino acid sequence of the natural α-glucan phosphorylase is encoded by a nucleic acid molecule which hybridizes under stringent condition to a nucleic acid molecule consisting of a base sequence encoding an amino acid sequence selected from the group consisting of: a position 1 to position 916 of SEQ ID NO: 2; position 1 to position 912 of SEQ ID NO: 4; position 1 to position 893 of SEQ ID NO: 6; position 1 to position 939 of SEQ ID NO: 8; position 1 to position 962 of SEQ ID NO: 10; position 1 to position 971 of SEQ ID NO: 12; position 1 to position 983 of SEQ ID NO: 14; position 1 to position 928 of SEQ ID NO: 16; position 1 to position 951 of SEQ ID

NO: 18; position 1 to position 832 of SEQ ID NO: 20; position 1 to position 840 of SEQ ID NO: 22; position 1 to position 841 of SEQ ID NO: 24; position 1 to position 842 of SEQ ID NO: 26; position 1 to position 841 of SEQ ID NO: 28; and position 1 to position 838 of SEQ ID NO: 30.

5. The α-glucan phosphorylase having improved thermostability according to claim 1, wherein the natural α-glucan phosphorylase is a type L α-glucan phosphorylase, and has an amino acid residue which is different from that of the natural α-glucan phosphorylase in at least one position selected from the group consisting of: a position corresponding to position 4 in the motif sequence 1L; a position corresponding to position 4 in the motif sequence; 2 and a position corresponding to position 7 in the motif sequence 3L.

6. The α-glucan phosphorylase having improved thermostability according to claim 1, wherein the natural α-glucan phosphorylase is a type H α-glucan phosphorylase, and has an amino acid residue which is different from that of the natural α-glucan phosphorylase in at least one position selected from the group consisting of: a position corresponding to position 4 in the motif sequence 1H; a position corresponding to position 4 in the motif sequence 2; and a position corresponding to position 7 in the motif sequence 3H.

7. The α-glucan phosphorylase having improved thermostability according to claim 1, wherein an amino acid sequence of the natural α-glucan phosphorylase is selected from the group consisting of: position 1 to position 916 of SEQ ID NO: 2; position 1 to position 912 of SEQ ID NO:

4; position 1 to position 893 of SEQ ID NO: 6; position 1 to position 939 of SEQ ID NO: 8; position 1 to position 962 of SEQ ID NO: 10; position 1 to position 971 of SEQ ID NO: 12; position 1 to position 983 of SEQ ID NO: 14; position 1 to position 928 of SEQ ID NO: 16; position 1 to position 951 of SEQ ID NO: 18; position 1 to position 832 of SEQ ID NO: 20; position 1 to position 840 of SEQ ID NO: 22; position 1 to position 841 of SEQ ID NO: 24; position 1 to position 842 of SEQ ID NO: 26; position 1 to position 841 of SEQ ID NO: 28; and position 1 to position 838 of SEQ ID NO: 30.

8. The α-glucan phosphorylase having improved thermostability according to claim 1, wherein the natural α-glucan phosphorylase is derived from potato or Arabidopsis thaliana.

9. The α-glucan phosphorylase having improved thermostability according to claim 1, which has an amino acid residue which is different from an amino acid residue of the natural α-glucan phosphorylase in at least two positions selected from the group consisting of: a position corresponding to position 4 in the motif sequence 1L or a position corresponding to position 4 in the motif sequence 1H; a position corresponding to position 4 in the motif sequence 2; and a position corresponding to position 7 in the motif sequence 3L or a position corresponding to position 7 in the motif sequence 3H.

10. The α-glucan phosphorylase having improved thermostability according to claim 1, which has an amino acid residue which is different from an amino acid residue of the natural α-glucan phosphorylase in: a position corresponding to position 4 in the motif sequence 1L or a

position corresponding to position 4 in the motif sequence 1H; a position corresponding to position 4 in the motif sequence 2; and a position corresponding to position 7 in the motif sequence 3L or a position corresponding to position 7 in the motif sequence 3H.

11. The α-glucan phosphorylase having improved thermostability according to claim 1, wherein an amino acid residue at a position corresponding to position 4 in the motif sequence 1L or a position corresponding to position 4 in the motif sequence 1H is selected from the group consisting of I, L and V.

12. The α-glucan phosphorylase having improved thermostability according to claim 1, wherein an amino acid residue at a position corresponding to position 4 in the motif sequence 1L or a position corresponding to position 4 in the motif sequence 1H is selected from the group consisting of I and L.

13. The α-glucan phosphorylase having improved thermostability according to claim 1, wherein an amino acid residue at a position corresponding to position 4 in the motif sequence 2 is selected from the group consisting of A, C, D, E, G, H, I, L, M, F, S, T, V and Y.

14. The α-glucan phosphorylase having improved thermostability according to claim 1, wherein an amino acid residue at a position corresponding to position 4 in the motif sequence 2 is selected from the group consisting of C, G, S and V.

15. The α-glucan phosphorylase having improved

thermostability according to claim 1, wherein an amino acid residue at a position corresponding to position 7 in the motif sequence 3L or a position corresponding to position 7 in the motif sequence 3H is selected from the group consisting of C, I, L, V and W.

16. The α-glucan phosphorylase having improved thermostability according to claim 1, wherein an amino acid residue at a position corresponding to position 7 in the motif sequence 3L or a position corresponding to position 7 in the motif sequence 3H is selected from the group consisting of C, I, L and V.

17. The α-glucan phosphorylase having improved thermostability according to claim 1, wherein enzyme activity at 37°C of the α-glucan phosphorylase having improved thermostability after heated in a 20 mM citrate buffer (pH 6.7) at 60°C for 10 minutes is 30% or more of enzyme activity at 37°C of the α-glucan phosphorylase having improved thermostability, before the heating.

18. The α-glucan phosphorylase having improved thermostability according to claim 1, wherein enzyme activity of the α-glucan phosphorylase having improved thermostability at 37°C, after heating in a 20 mM citrate buffer (pH 6.7) at 65°C for 2 minutes, is 10% or more of enzyme activity of the α-glucan phosphorylase having improved thermostability at 37°C, before heating.

19. The α-glucan phosphorylase having improved thermostability according to claim 1, wherein storage stability thereof is improved as compared with the natural α-glucan phosphorylase.

20. A method for producing α-glucan phosphorylase having improved thermostability, comprising:

modifying a first nucleic acid molecule comprising a base sequence encoding a first α-glucan phosphorylase to obtain a second nucleic acid molecule comprising a modified base sequence;

making an expression vector comprising the second nucleic acid molecule;

introducing the expression vector into a cell to express an α-glucan phosphorylase having improved thermostability; and

recovering the expressed α-glucan phosphorylase having improved thermostability,

wherein the first α-glucan phosphorylase is derived from a plant,

the α-glucan phosphorylase having improved thermostability has an amino acid residue which is different from an amino acid residue of the first α-glucan phosphorylase in at least one position selected from the group consisting of:

a position corresponding to position 4 in a motif sequence 1L: H-A-E-F-T-P-V-F-S or a position corresponding to position 4 in a motif sequence 1H: H-A-Q-Y-S-P-H-F-S;

a position corresponding to position 4 in a motif sequence 2: A-L-G-N-G-G-L-G; and

a position corresponding to position 7 in a motif sequence 3L: R-I-V-K-F-I-T-D-V or a position corresponding to position 7 in a motif sequence 3H: R-I-V-K-L-V-N-D-V; and

wherein the enzyme activity of the α-glucan phosphorylase having improved thermostability at 37°C, after heating in a 20 mM citrate buffer (pH 6.7) at 60°C for 10 minutes, is

20% or more of enzyme activity of the α-glucan phosphorylase having improved thermostability at 37°C, before heating.

21. The method according to claim 20, wherein an amino acid residue of the α-glucan phosphorylase having improved thermostability at: a position corresponding to position 4 in the motif sequence 1L or a position corresponding to position 4 in the motif sequence 1H; or a position corresponding to position 7 in the motif sequence 3L or a position corresponding to position 7 in the motif sequence 3H; is different from an amino acid residue of the first α-glucan phosphorylase.

22. The method according to claim 20, wherein the first α-glucan phosphorylase is a type L α-glucan phosphorylase, and has an amino acid residue which is different from that of the natural α-glucan phosphorylase in at least one position selected from the group consisting of: a position corresponding to position 4 in the motif sequence 1L; a position corresponding to position 4 in the motif sequence 2; and a position corresponding to position 7 in the motif sequence 3L.

23. The method according to claim 20, wherein the first α-glucan phosphorylase is a type H α-glucan phosphorylase, and has an amino acid residue which is different from that of the natural α-glucan phosphorylase in at least one position selected from the group consisting of: a position corresponding to position 4 in the motif sequence 1H; a position corresponding to position 4 in the motif sequence 2; and a position corresponding to position 7 in the motif sequence 3H.

24. The method according to claim 20, wherein the first α-glucan phosphorylase is derived from potato or Arabidopsis thaliana.

25. A nucleic acid molecule comprising a base sequence encoding the α-glucan phosphorylase having improved thermostability according to claim 1.

26. A vector comprising the nucleic acid molecule according to claim 25.

27. A cell comprising the nucleic acid molecule according to claim 25.

28. A method of synthesizing a glucan, comprising reacting a reaction solution containing the α-glucan phosphorylase having improved thermostability according to claim 1, a sucrose phosphorylase, sucrose, a primer, and inorganic phosphoric acid or glucose-1-phosphate to produce a glucan.

29. The method according to claim 28, wherein the reaction is performed at a temperature of 60°C to 75°C.

30. A method of synthesizing a glucan, comprising reacting a reaction solution containing the α-glucan phosphorylase having improved thermostability according to claim 1, a primer, and glucose-1-phosphate to produce a glucan.

31. The method according to claim 30, wherein the reaction is performed at a temperature of 60°C to 75°C.

32. A method of synthesizing glucose-1-phosphate, comprising reacting a reaction solution containing α-glucan

phosphorylase having improved thermostability according to claim 1, a glucan and inorganic phosphoric acid to produce glucose-1-phosphate.

33. The method according to claim 32, wherein the reaction is performed at a temperature of 60°C to 75°C.

34. An α-glucan phosphorylase having improved thermostability, which is obtained by modifying a plant-derived natural α-glucan phosphorylase,

wherein the α-glucan phosphorylase having improved thermostability has an amino acid residue which is different from an amino acid residue of the natural α-glucan phosphorylase at;

a position corresponding to position 4 in a motif sequence 1L: H-A-E-F-T-P-V-F-S or a position corresponding to position 4 in a motif sequence 1H: H-A-Q-Y-S-P-H-F-S;

a position corresponding to position 4 in a motif sequence 2L: A-L-G-N-G-G-L-G; and

a position corresponding to position 7 in a motif sequence 3L: R-I-V-K-F-I-T-D-V or a position corresponding to position 7 in a motif sequence 3H: R-I-V-K-L-V-N-D-V;

wherein the enzyme activity of the α-glucan phosphorylase having improved thermostability at 37°C, after heating in a 20 mM citrate buffer (pH 6.7) at 60°C for 10 minutes, is 20% or more of enzyme activity of the α-glucan phosphorylase having improved thermostability at 37°C, before heating, and

wherein the α-glucan phosphorylase having improved thermostability has ability to synthesize an amylose having a weight average molecular weight of 600 kDa or more.

35. An α-glucan phosphorylase having improved thermostability, which is obtained by modifying a natural

α-glucan phosphorylase,

wherein the natural α-glucan phosphorylase is derived from a plant,

the α-glucan phosphorylase having improved thermostability has an amino acid residue which is different from that of the natural α-glucan phosphorylase in at least one position selected from the group consisting of a position corresponding to phenylalanine at position 39 (F39), a position corresponding to asparagine at position 135 (N135) and a position corresponding to threonine at position 706 (T706) of an amino acid sequence of SEQ ID NO: 2, and

wherein the enzyme activity of the α-glucan phosphorylase having improved thermostability at 37°C, after heating in a 20 mM citrate buffer (pH 6.7) at 60°C for 10 minutes, is 20% or more of enzyme activity of the α-glucan phosphorylase having improved thermostability at 37°C, before heating.

36. A method for producing an α-glucan phosphorylase having improved thermostability, comprising:

modifying a first nucleic acid molecule comprising a base sequence encoding first α-glucan phosphorylase to obtain a second nucleic acid molecule comprising a modified base sequence;

making an expression vector comprising the second nucleic acid molecule;

introducing the expression vector into a cell to express α-glucan phosphorylase having improved thermostability; and

recovering the expressed α-glucan phosphorylase having improved thermostability,

wherein the first α-glucan phosphorylase is derived from a plant,

the α-glucan phosphorylase having improved thermostability has an amino acid residue which is different

from an amino acid residue of the first α-glucan phosphorylase in at least one position selected from the group consisting of: a position corresponding to phenylalanine at position 39 (F39); a position corresponding to asparagine at position 135 (N135); and a position corresponding to threonine at position 706 (T706) of an amino acid sequence of SEQ ID NO: 2, and

wherein the enzyme activity of the α-glucan phosphorylase having improved thermostability at 37°C, after heating in a 20 mM citrate buffer (pH 6.7) at 60°C for 10 minutes, is 20% or more of enzyme activity of the α-glucan phosphorylase having improved thermostability at 37°C, before heating.

37. A method of synthesizing a glucan, comprising reacting a reaction solution containing; the α-glucan phosphorylase having improved thermostability according to claim 35; a sucrose phosphorylase; sucrose; a primer; and an inorganic phosphoric acid or glucose-1-phosphate; to produce a glucan.

38. A method of synthesizing a glucan, comprising reacting a reaction solution containing the α-glucan phosphorylase having improved thermostability according to claim 35, a primer, and glucose-1-phosphate, to produce a glucan.

39. A method of synthesizing glucose-1-phosphate, comprising reacting a reaction solution containing the α-glucan phosphorylase having improved thermostability according to claim 35, a glucan and an inorganic phosphoric acid to produce glucose-1-phosphate.

40. An α-glucan phosphorylase having improved thermostability, which is obtained by modifying a plant-derived natural α-glucan phosphorylase,

wherein the α-glucan phosphorylase having improved thermostability has an amino acid residue which is different from an amino acid residue of the natural α-glucan phosphorylase in at least one position selected from the group consisting of: a position corresponding to phenylalanine at position 39 (F39); a position corresponding to asparagine at position 135 (N135); and a position corresponding to threonine at position 706 (T706) of an amino acid sequence of SEQ ID NO: 2,

wherein the enzyme activity of the α-glucan phosphorylase having improved thermostability at 37°C, after heating in a 20 mM citrate buffer (pH 6.7) at 60°C for 10 minutes, is 20% or more of enzyme activity of the α-glucan phosphorylase having improved thermostability at 37°C, before heating, and

wherein the α-glucan phosphorylase having improved thermostability has ability to synthesize amylose having a weight average molecular weight of 600 kDa or more.